Introduction: EGFR-TIKs showed about twenty percent of response rate in refractory non-small cell lung cancer. Clinical trials of cytotoxic drugs and EGFR-TIKs failed to show improved survival compared to platinum based doublets. An antagonism between EGFR-TKIs and cytotoxic chemotherapy drugs was raised as a possible explanation for the negative results.

Materials and Methods: The antiproliferative effects and cell cycle distributions after treatments with EGFR-TKIs(gefitinib and erlotinib) and cytotoxic drugs(Docetaxel, Paclitaxel, Gemcitabine) were studied using a cell line(NCI-H1975, adenocarcinoma of lung) harboring T790M mutation in exon 20 of EGFR gene. The cell viability assay and cell cycle analysis were performed with MTT assay and flow cytometry. EGFR-TKIs and cytotoxic drugs were treated in different sequences to observe sequence dependent effect. Calcusyn software(Biosoft, Cambridge, UK) was used to calculate combination index(CI).

Results: Various combinations of cytotoxic drugs and EGFR-TKIs showed different antiproliferative effects on NCI-H1975 cell line. Antagonisms(CI>1) were observed when EGFR-TKIs were treated before cytotoxic drugs(EC sequence), while synergisms(CI<1) were observed when cytotoxic drugs were pre-treated before EGFR-TKIs(CE sequence). Treatment in EC sequence arrested the cells in G0/G1 phase and decreased the apoptotic fraction. However, treatment in CE sequence arrested the cells in G2/M phase and higher fractions of apoptotic cell death were observed.

Conclusion: To combine EGFR-TKIs and cytotoxic drugs, sequence dependent anti-proliferative effects should be considered.

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Galectin-9 in stroma is a better prognostic indicator in lung cancer -Tissue Microarray Analysis

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Galectin-9 is a member of the β-galactoside-binding galectin family proteins associated with diverse biological processes, such as apoptosis, cell aggregation and eosinophilic chemoattraction. Some reports described that galectin-9 was a possible prognostic factor in breast cancer and melanoma.

We have found stromal spindle cells are occasionally positive for galectin-9 in our previous study (data not shown). Herein, we investigated its clinicopathological significance using lung cancer tissue microarray (TMA). We immunohistochemically examined the expression of galectin-9 in lung cancer using TMA containing samples from 400 surgical cases. Cancerous stroma was microscopically recognized in 183 cases (109 adenocarcinoma, 70 squamous cell carcinoma and 4 adenosquamous cell carcinoma cases).

Total of 24.6 % cases (45/183) showed galectin-9 expression in stromal spindle cells. We examined the survival statistical significance of galectin-9 using the log-rank test, and Kaplan-Meier curves were plotted. Positive immunohistochemical staining with galectin-9 was associated with favorer survival for patients with lung cancer (5-year survival of 59.2% versus 31.3% p=0.0206).

Conclusion: Our data indicates that galectin-9 in cancerous stroma can be a better prognostic biomarker in lung cancer.

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Podoplanin expression in cancerous stroma is a poor prognostic marker- Tissue Microarray Analysis

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Podoplanin is a mucin-type glycoprotein and a noble lymphatic endothelial marker. Immunohistochemical staining against podoplanin is currently a useful tool to detect lymphatic involvement of cancer cells, and is widely used in a routine pathological diagnosis. By observation of daily cases, we have noticed stromal spindle cells are occasionally positive for podoplanin. To confirm its presence and to investigate its clinical significance, we immunohistochemically examined podoplanin expression using several monoclonal antibodies and tissue microarrays.

We found that stromal podoplanin expression in adenocarcinoma was significantly associated with poorer prognosis ($p<0.001$). The prognostic significance was still high after adjustment with stage, gender, age, and histological differentiation ($p<0.001$). The expression was associated with differentiation and tended to associate with nodal metastasis. Also we immunohistochemically examined with 14 common cancer types and found that podoplanin expression was significantly associated with nodal metastasis ($p<.01$). Our data indicates that podoplanin expression in cancerous stromal cells may play a critical role in lymphatic invasion of cancer cells to determine patients' survival.

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Increased expression of survivin and its splice variants in non-small cell lung carcinoma

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Background: Apoptosome pathway dysfunction in tumor cells may account for their apoptosis resistance and there is evidence that survivin (Sur) and some its alternative splice variants may be involved in regulation of this cell death pathway in malignant tumors. In this work, we studied the expression of transcripts encoding Sur and its splice variants Sur-3B, Sur-2B and Sur-ΔEx3 in non-small cell lung carcinoma (NSCLC) tissues and lung parenchyma from surgically treated patients and examined the impact of survivin gene promoter genotype at nucleotide -31 and of the smoking status of NSCLC patients on the expression level of the indicated Sur transcript variants.

Methods: The expression of mRNAs encoding Sur, Sur-3B, Sur-2B and Sur-ΔEx3 was quantitated by real time RT-PCR using transcript-specific oligonucleotide primers and TaqMan fluorogenic probes, and an input of total RNA isolated from NSCLC and lung tissues. The expression of Sur transcript variants was normalized against the expression of β-actin mRNA. Genotyping of the survivin gene promoter in NSCLC and lung tissues was performed by PCR amplification and DNA sequencing of the purified PCR product with nested primers.